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CHAPTER 26

Humoral Immune Response to Lipopolysaccharide Antigens of *Campylobacter jejuni*

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INTRODUCTION

Campylobacter jejuni and the closely related organism *Campylobacter coli* have been established as among the most common bacterial causes of acute diarrheal disease of humans in developed and developing countries (5, 8). A wide variety of mammalian and avian species also are infected by those organisms and thus represent an important reservoir for transmission to humans. Development of vaccines against these organisms would therefore be worthwhile, but knowledge of their antigens is rudimentary (11, 12).

Epidemiologic and experimental data indicate that immunity to *C. jejuni* may be acquired following one or more infections (1, 6, 26). However, because these organisms are serologically diverse (19, 20), the nature of the protective antigens has not been immediately obvious. One approach to defining protective antigens is to determine which bacterial components are recognized by the host during infection (4, 23). Although such antigens are not necessarily protective, this approach nevertheless provides a first approximation of potential vaccine candidates.

We have been studying the humoral immune response to *Campylobacter* antigens in persons with acute diarrheal illnesses in the United States (22) in our search to define group-specific antigens which may have potential as vaccine candidates. One group of antigens studied, the proteins that are extracted from *C. jejuni* at low pH, are the subjects of several other reports (3, 6, 22). In the present chapter, we will describe humoral responses to the lipopolysaccharide (LPS) molecules of *C. jejuni*.

The LPS molecules of *C. jejuni* and *C. coli* are sufficiently diverse that typing schemes which rely on their heterogeneity have been developed (19); more than 50 different O types have been described (19, 20). However, our previous work has shown that *C. jejuni* LPS molecules possess both strain-specific epitopes and epitopes that are shared among campylobacters (23). LPS molecules of gram-negative bacteria are often immunodominant, and therefore their components have the potential to be used for serodiagnosis of infection (9) or to be considered for inclusion in vaccine formulations. Although *C. jejuni* LPS molecules contain epitopes conserved in other gram-negative organisms (24), other antigenic (23) and structural (10, 21) features suggest that they may be useful for serodiagnosis. Therefore, it is reasonable to question whether persons who are naturally infected with *Campylobacter* species show humoral immune responses to the LPS from homologous organisms (same O type) or from heterologous organisms (different O type). The response to heterologous organisms can be considered to be a response to group antigens.

DISTRIBUTION OF THE O ANTIGENS AMONG THE *CAMPYLOBACTER* ISOLATES

We first sought to determine whether *C. jejuni* and *C. coli* strains isolated from patients with sporadic cases of diarrhea (22) represented

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a broad distribution of O antigens (heat stable) so that we could study immune response to both homologous and heterologous LPSs. The 22 strains which had been studied were phenotypically characterized by using standard criteria (18). Ten different heat-labile antigens were represented, and five strains were nontypable (22). The *Campylobacter* isolates were then serotyped for heat-stable antigens by the Penner method as previously described (19, 20), and 12 different O types were found. For 10 strains, two or more O types were identified. Thus, in total, 37 O-type antigens were represented among these 22 strains. Six (27.2%) strains each had O:4 or O:5 antigenic determinants, and one strain had both group antigens. Other antigens identified (and the number of strains carrying them) were as follows: O:13 ($n = 5$); O:21 ($n = 4$); O:16 ($n = 3$); O:29, O:18, O:2, and O:3 ($n = 2$); O:38, O:41, and O:54 ($n = 1$). To study responses to both homologous and heterologous LPS antigens, we then prepared LPS from *C. jejuni* strains with O:4 and O:5 antigens, which were the antigens most commonly identified in the patient isolates. For comparison, we studied responses to O:1 and O:NT antigens, which represented unrelated *C. jejuni* LPS antigens.

CHARACTERISTICS OF THE LPS PREPARATIONS

These LPS molecules were prepared from each of four *C. jejuni* strains by the hot phenol-water method of Westphal and Jann (27), with subsequent purification steps performed as described by Hanson and Phillips (14). Each LPS preparation underwent a second phenolic extraction so that protein contamination was minimized, as previously described. Protein concentrations were measured by the method of Markwell et al. (17). For determination of the 2-keto-3-deoxyoctonate (KDO) concentration of the fractions, the thiobarbituric acid method described by Keleti and Lederer (16) was used, as previously described (21). Polyacrylamide gel electrophoresis (PAGE) was performed with slab gels as previously described (21). After electrophoresis, gels were fixed and LPS was resolved with a silver stain by the method of Hitchcock and Brown (15).

We found that for whole cells of all four strains, the ratio of KDO to protein (ratio A) was similar (Table 1). The ratio of KDO to protein in the LPS preparations (ratio B) ranged from 2.27 to 4.27. The ratio B/A ranged from 349.2 to 574.1, indicating that there had been a substantial decrease in the level of protein contamination of the preparations. In each case, protein contamination of the LPS preparation was <3%. By PAGE, each preparation appeared characteristic for *C. jejuni* LPS, with a core component migrating at less than 14,000 molecular weight and no bands greater than 20,000 (Fig. 1). Since LPS O type is heterogeneous (20), these four preparations afforded an opportunity to examine the antigenicity of strain-specific features of *Campylobacter* species.

SEROCONVERSION TO *C. JEJUNI* LPS

We then asked whether seroconversion to any of the LPS antigens differentiated the *Campylobacter*-infected and uninfected persons. The enzyme-linked immunosorbent assay (ELISA) used in this study was a modification of one described previously (3). In brief, a purified LPS preparation from each of the four strains was diluted in carbonate buffer to 100 μg/ml and used as antigen to coat wells of polystyrene microtiter plates. The screening serum dilutions were 1:200 for immunoglobulin M (IgM), 1:100 for IgG, and 1:50 for IgA measurements as de-

TABLE 1. Characteristics of LPS preparations extracted from four *C. jejuni* strains

Strain	O type[a]	Content (% of dry cell wt) of: KDO	Protein	Ratio A[b]	Content (% of dry LPS wt) of: KDO	Protein	Ratio B[b]	Ratio B/A
85-329	4	0.13	20	0.0065	5.9	2.6	2.27	349.2
85-360	5	0.14	22	0.0064	6.8	2.9	2.34	365.6
81-93	1	0.17	21	0.0081	4.7	1.1	4.27	527.2
79-193	NT[c]	0.15	26	0.0058	5.0	1.5	3.33	574.1

[a] By serotyping based on heat-stable antigens, as developed by Penner and Hennessy (20).
[b] Ratios A and B are defined in the text.
[c] NT, nontypable.

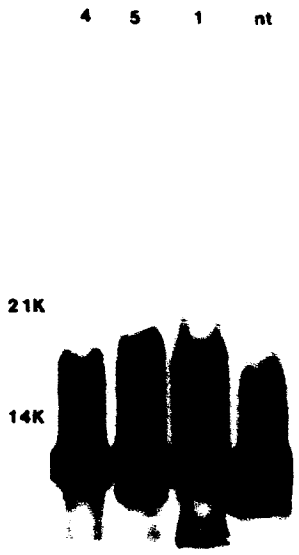

FIGURE 1. PAGE (15% acrylamide) of purified LPS preparations from four *C. jejuni* strains as resolved by silver stain. Preparations are as described in the text and represent LPS of types O:4, O:5, O:1, and O:NT (nontypable). Numbers at left refer to protein molecular weight standards simultaneously electrophoresed on the same gel.

termined by checkerboard titrations. Peroxidase conjugates of goat anti-human IgG diluted 1:4,000, IgA diluted 1:1,000, and IgM diluted 1:500 were used. Twenty-nine patients who were diagnosed as having acute (less than 1 week) inflammatory enteritis, based on an appropriate clinical history and the finding of fecal leukocytes in stool specimens, were included in the main study, as originally described (22). Twenty-two of these patients had enteritis due to *Campylobacter* species (17 *C. jejuni* and 5 *C. coli*). Another seven patients were infected with other enteric pathogens (two each with *Salmonella* or *Shigella* species) or no pathogen was identified (22); these seven represented the control group of persons with acute enteritis who were not infected with *Campylobacter* species. From each of the 29 patients we examined both an acute-phase serum sample obtained at the time of presentation of acute diarrheal illness and a convalescent-phase serum sample obtained 3 to 4 weeks later. Seroconversion was defined as a 50% increase in optical density between the acute- and convalescent-phase serum samples, as previously described (22). Of the seven persons who were not infected with *Campylobacter* species, none seroconverted to any of the four LPS preparations in any Ig class. In contrast, among the 22 persons infected with a *Campylobacter* species, seroconversion in one or more Ig classes occurred in 11, 12, 7, and 11 when the O-type 4, 5, 1, or NT LPS preparations, respectively, were used. In total, 17 (77.3%) of the 22 persons seroconverted to at least one LPS preparation in any Ig class. Compared with the response of the persons known to be uninfected, each of these differences was significant ($P < 0.02$; Fisher's exact test).

Because of the central role of IgA in mucosal immunity, the results of the studies of the IgA response in serum are highlighted (Fig. 2). There was essentially no serologic response among the persons without *Campylobacter* infection to any of the four *C. jejuni* LPS preparations. In contrast, among the infected persons, there was a significant ($P < 0.05$) rise in IgA levels directed toward each of the four LPS preparations.

Next, we focused on only a single LPS preparation, O:4, to examine the role of strain-specific and group-specific responses. The samples from the *Campylobacter*-infected persons showed significant increases in optical density values in the IgA, IgG, and IgM ELISAs toward the O:4 antigen (Fig. 3). As a group, the persons not infected with *Campylobacter* also had high antibody levels to the preparation, although the differences between acute- and convalescent-phase serum samples were not statistically significant.

Among the *Campylobacter*-infected persons, paired serum samples were available from 7 who were infected with an O:4-bearing strain and from 15 who were infected with heterologous *Campylobacter* strains. For the IgA and IgG classes, the optical density rose to an almost identical extent in the paired serum samples from the patients infected with homologous (O:4) and heterologous strains. However, patients infected with homologous strains showed a significant increase in the IgM response ($P < 0.01$), whereas those infected with heterologous strains did not (Fig. 4). A similar analysis of the data when the O:5 LPS (7 persons with homologous strains, 15 persons with heterologous strains) was used showed significant increases in IgG ($P = 0.02$) and IgM ($P = 0.05$) responses in persons infected with homologous strains; however, among persons infected with heterologous strains, only the IgA response was significant ($P < 0.008$).

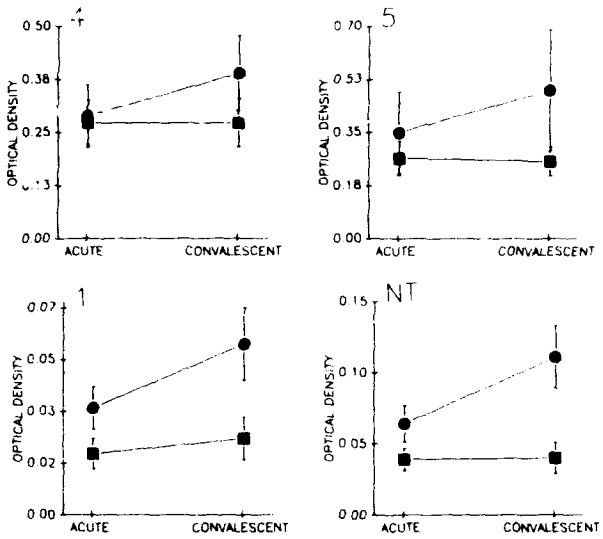

FIGURE 2. Serum IgA response to each of four *C. jejuni* LPS preparations by persons with inflammatory diarrhea infected (●; $n = 22$) or not infected (■; $n = 7$) with *Campylobacter* species. Optical densities in ELISAs directed against each LPS preparation were determined for both acute- and convalescent-phase serum samples.

SEROCONVERSION IN PATIENTS FROM WHOM NO PATHOGEN WAS IDENTIFIED

As part of this project, we examined serum from five patients from whom no pathogen was identified. Two of these patients had seroconverted to the group-specific *C. jejuni* protein antigens in each of the three Ig classes and were suspected of having been infected with *C. jejuni* (22). For comparison, serum was examined from three persons from whom no pathogen was identified and who had not responded to the protein antigens, suggesting that they had not been infected by a *Campylobacter* species. Assessing seroconversion to each of the four LPS preparations in each of the three Ig classes showed that serum samples from the two persons who recognized the protein antigens were positive in 11 (45.8%) of 24 assays, whereas none of 36 assays was positive for the three persons whose serum did not recognize the protein antigens ($P = 0.0001$; Fisher's exact test). These results further suggest that the two persons had been infected by a *Campylobacter* strain and that serology was probably more sensitive than culture for diagnosing this infection.

PERSPECTIVES

The presence of a serum antibody response to a particular antigen does not imply that that antigen elicits protective responses, nor that the serum antibody response measured is the mechanism of protection from reinfection. Nevertheless, the fact that naturally infected persons develop specific serum antibody responses to particular antigens suggests that such structures

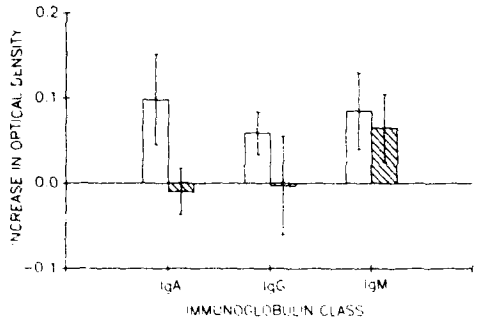

FIGURE 3. Serum antibody response to *C. jejuni* O:4 LPS in patients infected with *Campylobacter* species (open bars) or other pathogens (hatched bars). Differences in optical densities for acute- and convalescent-phase serum samples are compared for responses in the IgA, IgG, and IgM classes. For each Ig class, there was a significant difference ($P < 0.05$) between the *Campylobacter*-infected and noninfected persons.

could play a role in virulence or could have immunogenic potential.

We found that persons with natural *Campylobacter* infections showed serum antibody responses to purified *C. jejuni* LPS in each of the IgA, IgG, and IgM classes, whereas persons infected with other gram-negative pathogens or with acute diarrheal illness of unknown cause did not. Similarly, among persons experimentally infected with *C. jejuni*, a response to *C. jejuni* LPS also was observed (25). Although we examined preparations of *C. jejuni* LPS of four different O types, the responses to each of these were similar and there was little difference in response between persons infected with the homologous and heterologous LPS types. These phenomena suggest that the immunodominant *Campylobacter* LPS antigens are conserved among the four O types studied. In previous studies, we showed that by immunoblot *C. jejuni* LPS contained strain-specific antigens (23), as well as conserved antigens to which persons with heterologous infections respond. Alternatively, it is possible that the method of preparing the *C. jejuni* LPS altered or removed important distinguishing antigenic determinants; nevertheless, the PAGE profiles indicate that clear differences are apparent. However, under the assay conditions, no more than about half of the patients responded to any one preparation. Whether the difference in IgM and IgG response between those infected with strains of the homologous or heterologous LPS type is biologically significant must await further study.

Epidemiologic studies indicate that immunity to *C. jejuni* is acquired in areas in which infection is hyperendemic (2, 7, 13). Development of immunity implies that common immunogenic epitopes exist. Our studies suggest that some of these epitopes may be related to LPS-specific antigens. One implication of these investigations is that if an LPS constituent of a *Campylobacter* vaccine is considered, it may not be necessary to include a wide diversity of types. The results from our studies suggest that a single type might suffice. Similarly, an LPS preparation might be useful for serologic detection of *Campylobacter* infection. In fact, the results of this study are very similar to those from our previous work in which a *C. jejuni* surface protein-based preparation was used (22) and confirm that serology may be more sensitive than culture. It appears paradoxical that the LPS type can be used to differentiate *C. jejuni* and *C. coli* strains (19, 20) but that infected persons respond similarly to homologous and heterologous preparations. However, serotyping involves parenteral hyperimmunization of rabbits and absorption of the sera produced with heterologous strains. In contrast, since *Campylobacter* enteritis is a mucosal infection, antigen processing may be fundamentally different.

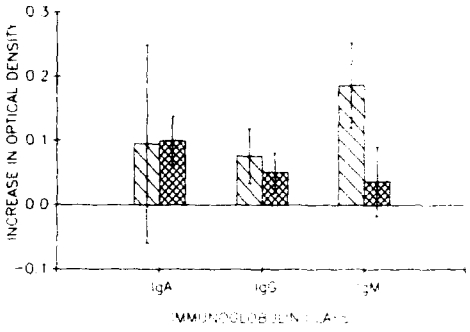

FIGURE 4. Serum antibody response to *C. jejuni* O:4 LPS among 7 persons infected with a *Campylobacter* strain bearing the O:4 antigen (homologous strain) (hatched bars) and 15 persons infected with a *Campylobacter* strain bearing heterologous LPS determinants (cross-hatched bars). Differences in optical densities for acute- and convalescent-phase serum samples are compared in the IgA, IgG, and IgM classes. For the IgM assay, there was a significant ($P = 0.01$) difference in response between the persons infected with homologous and heterologous strains.

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